TACTTCCAATCCATGTCTCGTGCCGCAGCCCGCTTTAAGATCCCGATGCCTGAGACCAAAGCGGACTTCGCGTTCCCGTCCCTGCGTGCTTTCTCTATTGTAGTAGCGCTGGACATGCAGCACGGTATTGGTGACGGCGAGTCTATTCCGTGGCGTGTACCGGAGGATATGACCTTTTTCAAGAATCAGACCACTCTGCTGCGCAACAAAAAACCGCCGACTGAGAAAAAACGCAATGCGGTTGTTATGGGCCGCAAAACTTGGGAATCTGTCCCTGTTAAATTCCGTCCACTCAAAGGTCGTCTGAACATCGTTCTGTCTTCTAAAGCGACTGTTGAGGAACTGCTCGCACCTCTCCCAGAAGGCCAGCGCGCAGCGGCAGCACAAGATGTTGTTGTGGTTAACGGTGGCCTGGCGGAAGCCCTCCGCCTCCTCGCGCGTCCGCTCTACTGTAGCTCTATCGAGACCGCCTACTGCGTAGGTGGTGCACAGGTTTACGCAGATGCCATGCTCTCCCCGTGCATCGAGAAGCTGCAGGAAGTATACCTGACTCGCATTTACGCGACGGCTCCGGCGTGCACTCGTTTCTTCCCGTTCCCTCCGGAAAACGCGGCGACTGCGTGGGATCTCGCGTCCTCTCAAGGTCGCCGTAAATCTGAAGCGGAAGGCCTGGAATTTGAAATCTGCAAATACGTTCCGCGTAATCACGAAGAGCGTCAGTATCTCGAACTCATCGACCGTATCATGAAAACTGGCATCGTGAAAGAAGACCGTACCGGTGTTGGCACCATCAGCCTGTTTGGTGCTCAGATGCGTTTCTCTCTGCGTGACAACCGTCTGCCACTGCTGACTACGAAACGTGTGTTCTGGCGTGGTGTTTGTGAGGAGCTGCTGTGGTTCCTCCGCGGTGAGACGTCCGCGCAACTCCTGGCCGACAAAGACATTCACATCTGGGACGGCAATGGTTCCCGCGAGTTCCTGGACTCTCGTGGCCTCACCGAGAACAAGGAGATGGACCTGGGTCCGGTGTACGGCTTTCAGTGGCGTCACTTCGGTGCGGACTACAAAGGTTTCGAAGCGAACTACGATGGCGAAGGTGTTGACCAGATCAAACTGATCGTTGAAACCATCAAAACCAACCCTAACGACCGTCGTCTCCTGGTAACCGCGTGGAACCCGTGCGCCCTGCAAAAGATGGCGCTCCCGCCGTGTCATCTGCTGGCGCAATTCTACGTGAACACCGACACCTCTGAACTGTCTTGCATGCTGTACCAGCGTTCTTGCGACATGGGCCTGGGCGTTCCTTTCAACATCGCGTCTTACGCGCTCCTGACCATTCTCATCGCGAAAGCCACGGGCCTGCGTCCGGGCGAACTGGTCCATACCCTGGGTGACGCGCATGTTTACCGCAACCACGTTGATGCGCTGAAGGCCCAGCTCGAACGTGTTCCACATGCATTCCCGACCCTGATTTTCAAAGAGGAACGTCAATACCTGGAGGACTATGAACTGACTGATATGGAAGTGATCGATTATGTACCACATCCGGCGATTAAGATGGAAATGGCTGTCTAACAGTAAAGGTGGATA

Forward Primer: TACTTCCAATCCATGTCTCGTGCCGCAGCCCGC

Reverse Primer: TATCCACCT**TTA**CTGTTAGACAGCCATTTCCATCTT

**Forward Primer:**

5’- TACTTCCAATCCATGTCTCGTGCCGCAGCCCGC -3’ \_33\_ bp

GC Content \_60.6\_%

0 mM Mg2+ Tm \_70.3\_°C 1.5 mM Mg2+ Tm \_76.4\_°C 2 mM Mg2+ Tm \_76.8\_°C

4 mM Mg2+ Tm \_77.5\_°C 6 mM Mg2+ Tm \_77.8\_°C

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**Reverse Primer:**

5’- AAGATGGAAATGGCTGTCTAACAGTAAAGGTGGATA -3’

**Reverse complement it:**

5’- TATCCACCTTTACTGTTAGACAGCCATTTCCATCTT -3’ \_36\_ bp

GC Content \_38.9\_%

0 mM Mg2+ Tm \_61.9\_°C 1.5 mM Mg2+ Tm \_69.7\_°C 2 mM Mg2+ Tm \_70.2\_°C

4 mM Mg2+ Tm \_71.2\_°C 6 mM Mg2+ Tm \_71.6\_°C

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Create a Virtual Plasmid:

**In WORD, paste**

1. Insert: your CDS with Lagger and Leader portions of the primers added to the ends

2. Accepting Vector: full pNIC-Bsa4 sequence (FASTA sequence of pNIC-Bsa4 is on Google Docs)

**In NEB Cutter online,**

Virtually cut your Insert with BsaI to determine how the ends will be on your insert

Then in another NEB Cutter window, load the sequence of the Accepting Vector

Virtually cut your Accepting Vector with BsaI to determine where your GOI will fit inside the pNIC-Bsa4 sequence (HINT: show as linear and use the zoom feature to see individual nucleotides)

**Back in WORD** - using these locations, paste together what your full plasmid should look like with the Gene inserted

Show this and highlight your gene and the start and stop codons.

Highlight where the 6xHIS tag is.

Save results of your Virtual Plasmid to the Google Docs and to your Wikispaces page).

Include your Name and Date in the filename and on the document itself.

**pNIC28-Bsa4 with Dihydrofolate Reductase-Thymidylate Synthase Insert**

TAATACGACTCACTATAGGGGAATTGTGAGCGGATAACAATTCCCCTCTAGAAATAATTTTGTTTAACTTTAAGAAGGAGATATACATATGCACCATCATCATCATCATTCTTCTGGTGTAGATCTGGGTACCGAGAACCTGTACTTCCAATCCATGTCTCGTGCCGCAGCCCGCTTTAAGATCCCGATGCCTGAGACCAAAGCGGACTTCGCGTTCCCGTCCCTGCGTGCTTTCTCTATTGTAGTAGCGCTGGACATGCAGCACGGTATTGGTGACGGCGAGTCTATTCCGTGGCGTGTACCGGAGGATATGACCTTTTTCAAGAATCAGACCACTCTGCTGCGCAACAAAAAACCGCCGACTGAGAAAAAACGCAATGCGGTTGTTATGGGCCGCAAAACTTGGGAATCTGTCCCTGTTAAATTCCGTCCACTCAAAGGTCGTCTGAACATCGTTCTGTCTTCTAAAGCGACTGTTGAGGAACTGCTCGCACCTCTCCCAGAAGGCCAGCGCGCAGCGGCAGCACAAGATGTTGTTGTGGTTAACGGTGGCCTGGCGGAAGCCCTCCGCCTCCTCGCGCGTCCGCTCTACTGTAGCTCTATCGAGACCGCCTACTGCGTAGGTGGTGCACAGGTTTACGCAGATGCCATGCTCTCCCCGTGCATCGAGAAGCTGCAGGAAGTATACCTGACTCGCATTTACGCGACGGCTCCGGCGTGCACTCGTTTCTTCCCGTTCCCTCCGGAAAACGCGGCGACTGCGTGGGATCTCGCGTCCTCTCAAGGTCGCCGTAAATCTGAAGCGGAAGGCCTGGAATTTGAAATCTGCAAATACGTTCCGCGTAATCACGAAGAGCGTCAGTATCTCGAACTCATCGACCGTATCATGAAAACTGGCATCGTGAAAGAAGACCGTACCGGTGTTGGCACCATCAGCCTGTTTGGTGCTCAGATGCGTTTCTCTCTGCGTGACAACCGTCTGCCACTGCTGACTACGAAACGTGTGTTCTGGCGTGGTGTTTGTGAGGAGCTGCTGTGGTTCCTCCGCGGTGAGACGTCCGCGCAACTCCTGGCCGACAAAGACATTCACATCTGGGACGGCAATGGTTCCCGCGAGTTCCTGGACTCTCGTGGCCTCACCGAGAACAAGGAGATGGACCTGGGTCCGGTGTACGGCTTTCAGTGGCGTCACTTCGGTGCGGACTACAAAGGTTTCGAAGCGAACTACGATGGCGAAGGTGTTGACCAGATCAAACTGATCGTTGAAACCATCAAAACCAACCCTAACGACCGTCGTCTCCTGGTAACCGCGTGGAACCCGTGCGCCCTGCAAAAGATGGCGCTCCCGCCGTGTCATCTGCTGGCGCAATTCTACGTGAACACCGACACCTCTGAACTGTCTTGCATGCTGTACCAGCGTTCTTGCGACATGGGCCTGGGCGTTCCTTTCAACATCGCGTCTTACGCGCTCCTGACCATTCTCATCGCGAAAGCCACGGGCCTGCGTCCGGGCGAACTGGTCCATACCCTGGGTGACGCGCATGTTTACCGCAACCACGTTGATGCGCTGAAGGCCCAGCTCGAACGTGTTCCACATGCATTCCCGACCCTGATTTTCAAAGAGGAACGTCAATACCTGGAGGACTATGAACTGACTGATATGGAAGTGATCGATTATGTACCACATCCGGCGATTAAGATGGAAATGGCTGTCTAACAGTAAAGGTGGATACGGATCCGAATTCGAGCTCCGTCGACAAGCTTGCGGCCGCACTCGAGCACCACCACCACCACCACTGAGATCCGGCTGCTAACAAAGCCCGAAAGGAAGCTGAGTTGGCTGCTGCCACCGCTGAGCAATAACTAGCATAACCCCTTGGGGCCTCTAAACGGGTCTTGAGGGGTTTTTTGCTGAAAGGAGGAACTATATCCGGATTGGCGAATGGGACGCGCCCTGTAGCGGCGCATTAAGCGCGGCGGGTGTGGTGGTTACGCGCAGCGTGACCGCTACACTTGCCAGCGCCCTAGCGCCCGCTCCTTTCGCTTTCTTCCCTTCCTTTCTCGCCACGTTCGCCGGCTTTCCCCGTCAAGCTCTAAATCGGGGGCTCCCTTTAGGGTTCCGATTTAGTGCTTTACGGCACCTCGACCCCAAAAAACTTGATTAGGGTGATGGTTCACGTAGTGGGCCATCGCCCTGATAGACGGTTTTTCGCCCTTTGACGTTGGAGTCCACGTTCTTTAATAGTGGACTCTTGTTCCAAACTGGAACAACACTCAACCCTATCTCGGTCTATTCTTTTGATTTATAAGGGATTTTGCCGATTTCGGCCTATTGGTTAAAAAATGAGCTGATTTAACAAAAATTTAACGCGAATTTTAACAAAATATTAACGTTTACAATTTCAGGTGGCACTTTTCGGGGAAATGTGCGCGGAACCCCTATTTGTTTATTTTTCTAAATACATTCAAATATGTATCCGCTCATGAATTAATTCTTAGAAAAACTCATCGAGCATCAAATGAAACTGCAATTTATTCATATCAGGATTATCAATACCATATTTTTGAAAAAGCCGTTTCTGTAATGAAGGAGAAAACTCACCGAGGCAGTTCCATAGGATGGCAAGATCCTGGTATCGGTCTGCGATTCCGACTCGTCCAACATCAATACAACCTATTAATTTCCCCTCGTCAAAAATAAGGTTATCAAGTGAGAAATCACCATGAGTGACGACTGAATCCGGTGAGAATGGCAAAAGTTTATGCATTTCTTTCCAGACTTGTTCAACAGGCCAGCCATTACGCTCGTCATCAAAATCACTCGCATCAACCAAACCGTTATTCATTCGTGATTGCGCCTGAGCGAGACGAAATACGCGATCGCTGTTAAAAGGACAATTACAAACAGGAATCGAATGCAACCGGCGCAGGAACACTGCCAGCGCATCAACAATATTTTCACCTGAATCAGGATATTCTTCTAATACCTGGAATGCTGTTTTCCCGGGGATCGCAGTGGTGAGTAACCATGCATCATCAGGAGTACGGATAAAATGCTTGATGGTCGGAAGAGGCATAAATTCCGTCAGCCAGTTTAGTCTGACCATCTCATCTGTAACATCATTGGCAACGCTACCTTTGCCATGTTTCAGAAACAACTCTGGCGCATCGGGCTTCCCATACAATCGATAGATTGTCGCACCTGATTGCCCGACATTATCGCGAGCCCATTTATACCCATATAAATCAGCATCCATGTTGGAATTTAATCGCGGCCTAGAGCAAGACGTTTCCCGTTGAATATGGCTCATAACACCCCTTGTATTACTGTTTATGTAAGCAGACAGTTTTATTGTTCATGACCAAAATCCCTTAACGTGAGTTTTCGTTCCACTGAGCGTCAGACCCCGTAGAAAAGATCAAAGGATCTTCTTGAGATCCTTTTTTTCTGCGCGTAATCTGCTGCTTGCAAACAAAAAAACCACCGCTACCAGCGGTGGTTTGTTTGCCGGATCAAGAGCTACCAACTCTTTTTCCGAAGGTAACTGGCTTCAGCAGAGCGCAGATACCAAATACTGTCCTTCTAGTGTAGCCGTAGTTAGGCCACCACTTCAAGAACTCTGTAGCACCGCCTACATACCTCGCTCTGCTAATCCTGTTACCAGTGGCTGCTGCCAGTGGCGATAAGTCGTGTCTTACCGGGTTGGACTCAAGACGATAGTTACCGGATAAGGCGCAGCGGTCGGGCTGAACGGGGGGTTCGTGCACACAGCCCAGCTTGGAGCGAACGACCTACACCGAACTGAGATACCTACAGCGTGAGCTATGAGAAAGCGCCACGCTTCCCGAAGGGAGAAAGGCGGACAGGTATCCGGTAAGCGGCAGGGTCGGAACAGGAGAGCGCACGAGGGAGCTTCCAGGGGGAAACGCCTGGTATCTTTATAGTCCTGTCGGGTTTCGCCACCTCTGACTTGAGCGTCGATTTTTGTGATGCTCGTCAGGGGGGCGGAGCCTATGGAAAAACGCCAGCAACGCGGCCTTTTTACGGTTCCTGGCCTTTTGCTGGCCTTTTGCTCACATGTTCTTTCCTGCGTTATCCCCTGATTCTGTGGATAACCGTATTACCGCCTTTGAGTGAGCTGATACCGCTCGCCGCAGCCGAACGACCGAGCGCAGCGAGTCAGTGAGCGAGGAAGCGGAAGAGCGCCTGATGCGGTATTTTCTCCTTACGCATCTGTGCGGTATTTCACACCGCATATATGGTGCACTCTCAGTACAATCTGCTCTGATGCCGCATAGTTAAGCCAGTATACACTCCGCTATCGCTACGTGACTGGGTCATGGCTGCGCCCCGACACCCGCCAACACCCGCTGACGCGCCCTGACGGGCTTGTCTGCTCCCGGCATCCGCTTACAGACAAGCTGTGACCGTCTCCGGGAGCTGCATGTGTCAGAGGTTTTCACCGTCATCACCGAAACGCGCGAGGCAGCTGCGGTAAAGCTCATCAGCGTGGTCGTGAAGCGATTCACAGATGTCTGCCTGTTCATCCGCGTCCAGCTCGTTGAGTTTCTCCAGAAGCGTTAATGTCTGGCTTCTGATAAAGCGGGCCATGTTAAGGGCGGTTTTTTCCTGTTTGGTCACTGATGCCTCCGTGTAAGGGGGATTTCTGTTCATGGGGGTAATGATACCGATGAAACGAGAGAGGATGCTCACGATACGGGTTACTGATGATGAACATGCCCGGTTACTGGAACGTTGTGAGGGTAAACAACTGGCGGTATGGATGCGGCGGGACCAGAGAAAAATCACTCAGGGTCAATGCCAGCGCTTCGTTAATACAGATGTAGGTGTTCCACAGGGTAGCCAGCAGCATCCTGCGATGCAGATCCGGAACATAATGGTGCAGGGCGCTGACTTCCGCGTTTCCAGACTTTACGAAACACGGAAACCGAAGACCATTCATGTTGTTGCTCAGGTCGCAGACGTTTTGCAGCAGCAGTCGCTTCACGTTCGCTCGCGTATCGGTGATTCATTCTGCTAACCAGTAAGGCAACCCCGCCAGCCTAGCCGGGTCCTCAACGACAGGAGCACGATCATGCGCACCCGTGGGGCCGCCATGCCGGCGATAATGGCCTGCTTCTCGCCGAAACGTTTGGTGGCGGGACCAGTGACGAAGGCTTGAGCGAGGGCGTGCAAGATTCCGAATACCGCAAGCGACAGGCCGATCATCGTCGCGCTCCAGCGAAAGCGGTCCTCGCCGAAAATGACCCAGAGCGCTGCCGGCACCTGTCCTACGAGTTGCATGATAAAGAAGACAGTCATAAGTGCGGCGACGATAGTCATGCCCCGCGCCCACCGGAAGGAGCTGACTGGGTTGAAGGCTCTCAAGGGCATCGGTCGAGATCCCGGTGCCTAATGAGTGAGCTAACTTACATTAATTGCGTTGCGCTCACTGCCCGCTTTCCAGTCGGGAAACCTGTCGTGCCAGCTGCATTAATGAATCGGCCAACGCGCGGGGAGAGGCGGTTTGCGTATTGGGCGCCAGGGTGGTTTTTCTTTTCACCAGTGAGACGGGCAACAGCTGATTGCCCTTCACCGCCTGGCCCTGAGAGAGTTGCAGCAAGCGGTCCACGCTGGTTTGCCCCAGCAGGCGAAAATCCTGTTTGATGGTGGTTAACGGCGGGATATAACATGAGCTGTCTTCGGTATCGTCGTATCCCACTACCGAGATATCCGCACCAACGCGCAGCCCGGACTCGGTAATGGCGCGCATTGCGCCCAGCGCCATCTGATCGTTGGCAACCAGCATCGCAGTGGGAACGATGCCCTCATTCAGCATTTGCATGGTTTGTTGAAAACCGGACATGGCACTCCAGTCGCCTTCCCGTTCCGCTATCGGCTGAATTTGATTGCGAGTGAGATATTTATGCCAGCCAGCCAGACGCAGACGCGCCGAGACAGAACTTAATGGGCCCGCTAACAGCGCGATTTGCTGGTGACCCAATGCGACCAGATGCTCCACGCCCAGTCGCGTACCGTCTTCATGGGAGAAAATAATACTGTTGATGGGTGTCTGGTCAGAGACATCAAGAAATAACGCCGGAACATTAGTGCAGGCAGCTTCCACAGCAATGGCATCCTGGTCATCCAGCGGATAGTTAATGATCAGCCCACTGACGCGTTGCGCGAGAAGATTGTGCACCGCCGCTTTACAGGCTTCGACGCCGCTTCGTTCTACCATCGACACCACCACGCTGGCACCCAGTTGATCGGCGCGAGATTTAATCGCCGCGACAATTTGCGACGGCGCGTGCAGGGCCAGACTGGAGGTGGCAACGCCAATCAGCAACGACTGTTTGCCCGCCAGTTGTTGTGCCACGCGGTTGGGAATGTAATTCAGCTCCGCCATCGCCGCTTCCACTTTTTCCCGCGTTTTCGCAGAAACGTGGCTGGCCTGGTTCACCACGCGGGAAACGGTCTGATAAGAGACACCGGCATACTCTGCGACATCGTATAACGTTACTGGTTTCACATTCACCACCCTGAATTGACTCTCTTCCGGGCGCTATCATGCCATACCGCGAAAGGTTTTGCGCCATTCGATGGTGTCCGGGATCTCGACGCTCTCCCTTATGCGACTCCTGCATTAGGAAGCAGCCCAGTAGTAGGTTGAGGCCGTTGAGCACCGCCGCCGCAAGGAATGGTGCATGCAAGGAGATGGCGCCCAACAGTCCCCCGGCCACGGGGCCTGCCACCATACCCACGCCGAAACAAGCGCTCATGAGCCCGAAGTGGCGAGCCCGATCTTCCCCATCGGTGATGTCGGCGATATAGGCGCCAGCAACCGCACCTGTGGCGCCGGTGATGCCGGCCACGATGCGTCCGGCGTAGAGGATCGAGATCTCGATCCCGCGAAAT